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15. Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, by NSF grant AER77-00879, and by U.S. Department of Agriculture grant 416-15-77. We thank S. Maier, A. Wopat, D. Noel, and L. Hansen for help with this project.
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Abnormal Auditory Evoked Potentials in Early Infancy Malnutrition

Abstract. *Computer-averaged auditory evoked potentials were found to be abnormal in infants hospitalized because of severe malnutrition (marasmus). They improved as the infants' somatic growth improved during the course of treatment, but were still deviant at the time of discharge from the hospital and at subsequent outpatient follow-up. Abnormalities in evoked potentials may reflect a long-lasting effect of malnutrition on brain function.*

Chronic malnutrition during infancy causes growth failure which is accompanied by many signs and symptoms of developmental delay. There is substantial but still controversial evidence that brain dysfunction caused by early malnutrition may, in part, be irreversible (*1*-*3*). The issue is difficult to resolve because of limitations in the traditional methods for evaluating perceptual and cognitive functions during infancy.

Sensory evoked brain potentials provide measures of cerebral function that are correlated with brain maturation and development (*4*-*6*). They are sensitive to cognitive and perceptual variables (*7*), and they are abnormal in some patients with cerebral dysfunction (*8*). Electroencephalographic (EEG) abnormalities associated with chronic malnutrition have been reported (*9*), and in experimental animals abnormalities of evoked potentials have also been noted (*10*). We have made a systematic study of evoked potentials in human malnutrition, and have found that they are abnormal in malnourished infants. Although the abnormalities improve during nutritional therapy, they continue to be significantly different from the evoked potentials of normal children.

We recorded evoked potentials from 13 boys and 13 girls (3 to 12 months of age) admitted to the nutrition service of a pediatric hospital in Mexico because of severe protein-calorie malnutrition (marasmus). The weight of each infant was less than 60 percent of the expected weight for chronological age. Length was also grossly deviant (*11*). Healthy infants, 25 boys and 21 girls, from the hospital employees' Day Care Center served as control subjects. The mean age and age range for the control subjects matched that of the patients. Children with histories of low birth weight (less than 2500 g) were excluded from both samples (*12*), as were those with neurological dysfunction and congenital abnormalities.

Evoked potentials were obtained from patients (i) shortly after they were admitted to the hospital but after acute metabolic abnormalities and infections had been treated, (ii) at the start of steady weight gain, (iii) when they attained a weight of 5 kg, (iv) just prior to discharge, and (v) at follow-up sessions 2 to 12 months after discharge. Discharge occurred when the patient was in good physical health and had attained a weight-age ratio equal to his or her height-age ratio (*13*). For each control subject we used evoked potentials obtained during one or two recording sessions.

A test session consisted of the presentation of many sets of 100 visual and auditory stimuli during the recording of the EEG (bandpass: 0.7 to 70 Hz) from standard placements (*14*). Averaged evoked potentials for a 1280-msec poststimulus period were derived with the use of a waveform averager (Northern Scientific model 575; sampling rate, one per 5 msec). We report here the results for evoked potentials obtained during unsedated daytime sleep (*15, 16*) in response to 100 clicks (65 dB, sensation level) presented at a rate of one per 2.5 seconds through a loudspeaker and recorded from an electrode at the vertex of the scalp (C_z) referred to joined mastoids. A total of 183 such evoked potentials were collected from the patient group and 68 from the control subjects. Examples are shown in Fig. 1. Latencies and associated amplitudes were measured for the most prominent evoked potential components, that is, N_1, P_2, N_2, and P_3. Approximate latencies of these components were 100, 200, 400, and 700 msec, respectively. In order to quantify the relative normality or abnormality of each evoked potential, an evoked potential index (EPI) was derived. Each EPI was the sum of the absolute values for the deviations (in standard deviations, S.D.'s) from mean normal values of the four peak latencies and corresponding three peak-to-peak amplitudes. Normal means and S.D.'s were available from a previous study of 130 normal children in seven age groups from 0.5 to 36 months of age (*17*). Deviations greater than 2 S.D.'s were scored as 2, resulting in a scale which varied from 0 (all components within 1 S.D. of the normal means) to 14 (all components 2 or more S.D.'s from the means). The evoked potentials in the present study had EPI's that ranged from 0 to 12.

The mean EPI for all evoked potentials from the marasmic subjects was 5.6 ± 2.5. For the control subjects this value was 3.5 ± 1.7. On admission, the mean EPI of the patients was 6.4 (Fig. 2). As a measure after rehabilitation, the

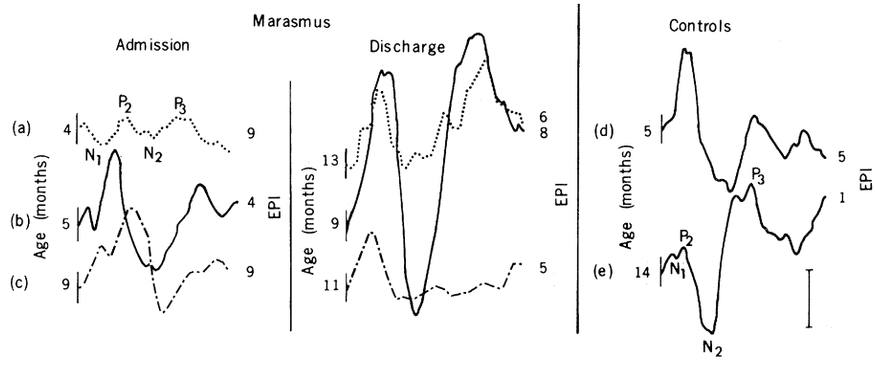

Fig. 1. Averaged evoked potentials to 65-dB clicks (N = 100, interstimulus interval, 2.5 seconds) from three malnourished patients (*a*), (*b*), and (*c*), and two control subjects (*d*) and (*e*). Upward deflection signifies positivity at the vertex (C_z) with respect to a linked mastoid reference. The duration of the tracing is 1 second; the stimulus occurred at the beginning. The calibration, which applies to all the evoked potentials, is 25 μV. The evoked potential index (*EPI*) is a measure of the deviation from normal values (*17*) of the labeled evoked potential components, with a higher score denoting greater deviance.

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weighted means of the discharge and follow-up EPI's was used. This value, 5.2, was found to be significantly lower than the admission EPI (Student's t-test, 2.12, d.f. = 121, $P < .05$). The EPI's for male and female control subjects were 3.5 and 3.7, respectively. For the male and female patients at admission the EPI's were 6.1 and 6.6; after rehabilitation they were 4.6 for males and 5.7 for the females. These last values suggest a sex difference in the EPI measure, although tests for significance did not confirm the effect (*18*). The EPI's of the rehabilitated marasmic children were compared to those of the control subjects. They were found to be significantly higher than those of the same-sex controls (males, $t = 2.72$, d.f. = 62, $P < .01$; females, $t = 5.66$, d.f. = 58, $P < .01$; Student's t-test).

Over the course of hospitalization and follow-up, the type as well as the degree of evoked potential abnormality changed as did background EEG and sleep patterns (*16*). Figure 3 indicates the direction of deviation from the normal means for each of the component peaks of the evoked potential. On admission, a frequent finding was long latency and low amplitude of the $P_2N_2P_3$ complex. Some female patients on admission showed very high amplitudes for N_1P_2 and P_2N_2. Very high amplitude for these waves was the most persistent morphological abnormality, and this abnormality seemed to be primarily a characteristic of the female patients. The mean Z scores for N_1P_2 illustrate this point. For the males on successive tests from admission to the second follow-up session, these scores were 0.6, 0.1, 2.4, 0.7, 0.8, and 0.5. The analogues values for females were 2.0, 4.2, 2.7, 3.4, 2.9, and 4.

At admission, males and females did not differ substantially in age, or in severity of malnutrition as indexed by height, weight, and head circumference, and both sexes improved on these measures during hospitalization at approximately the same rate. For the females only, it was found that short stature on admission was associated with a high discharge EPI. In the control subjects, there appeared to be an interaction of EPI with stature and sex analogous to that found in the patients. Ten male and four female controls were slightly below the third percentile in height (*19*). For the evoked potentials from the control males and females of normal stature, the mean EPI's were 3.2 ($N = 15$) and 3.0 ($N = 17$), respectively; for the males of shorter stature the mean was 3.7, but for the females of shorter stature it was 5.0 (*20*).

The auditory EPI's of malnourished subjects of both sexes remain higher than those of normal controls for as long as a year after therapeutic intervention. We do not know how long the high EPI's persist, or if high scores would be found in infants less severely malnourished. The fact that the short stature of some of the control subjects may represent an effect of chronic malnutrition, coupled with our finding that the EPI's of these children were higher than those of the taller children, leads us to believe that the exploration of evoked potentials as measures of cerebral dysfunction in less severe as well as more severe degrees of malnutrition would be worthwhile.

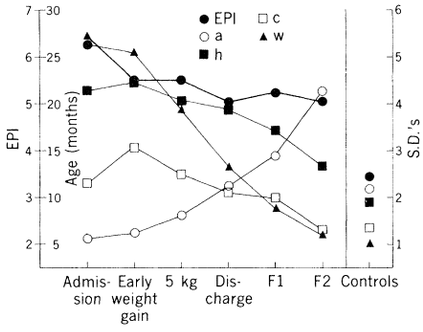

Fig. 2. Evoked potential index (*EPI*), age (*a*), height (*h*), weight (*w*), and head circumference (*c*) of malnourished patients at several stages during hospitalization and at subsequent follow-up examinations (*F1* and *F2*); and of control subjects. A high EPI indicates greater evoked potential abnormality. Height, weight, and head circumference are expressed in standard deviations (*S.D.'s*) below normal mean values (*11*).

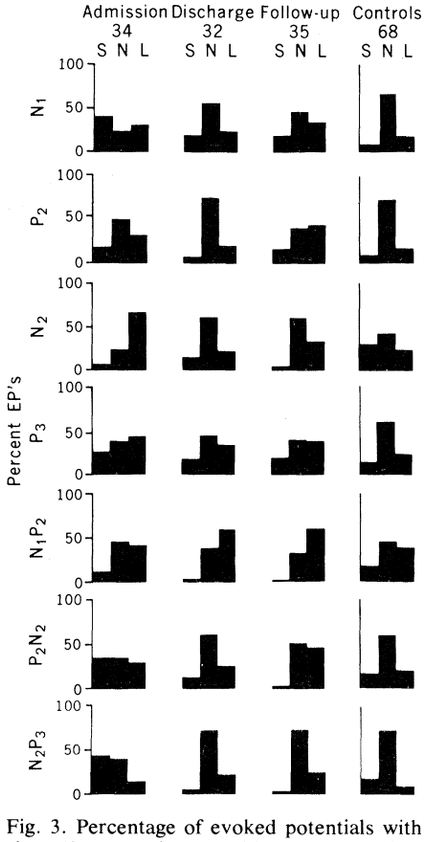

Fig. 3. Percentage of evoked potentials with short (*S*), normal (*N*), and long (*L*) latencies; and small (*S*), normal (*N*), and large (*L*) amplitudes for several components. Data for malnourished infants at several states of rehabilitation and for control subjects is given. The *S* and *L* classifications represent values more than 1 S.D. from norms for same-aged children (*17*).

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11. For grading the nutritional state of the child, the Gomez classification based on the relation between weight and age was used [F. R. Gomez, R. Ramos-Galvan, J. Cravioto, S. Frank, *Adv. Pediatr.* **7**, 131 (1955); R. Ramos-Galvan, J. Cravioto, A. Navarrete, *Bol. Med. Hosp. Infant. Mex.* **16**, 876 (1958), Spanish ed.]. These values were applied to the weight standards developed at the Harvard School of Public Health (HSPH) and published in Nelson *et al.* [W. E. Nelson, V. C. Vaughan III, R. J. McKay, Eds., *Textbook of Pediatrics* (Saunders, Philadelphia, ed.

10, 1976), pp. 39–48, and other editions]. Height measurements were also compared to the HSPH norms. The Composite International and Interracial Graphs compiled by G. Nellhaus [*Pediatrics* **41**, 106 (1968)] were used for head circumference. These references were chosen because they are widely utilized in pediatric growth measurements [see, for example, R. R. Puffer and C. V. Serrano, *Pan. Am. Health Organ. Sci. Publ. No. 262* (1973)]. They differ only slightly from other standards. See M. Behar [in *Malnutrition, Learning, and Behavior*, N. S. Scrimshaw and J. E. Gordon, Eds. (MIT Press, Cambridge, Mass., 1968), p. 83] for discussion of reference standards for physical growth.

12. Information concerning birthweight and maturity at birth, although available on all the subjects, was judged to be of doubtful reliability in some cases.
13. To determine the ratio of weight to age and height to age, the patient's height and weight are compared to the normal reference standards to ascertain the age at which the patient's measure would fall on the normal mean. The patient's height and weight ages were always below their chronological ages.
14. The methods and procedures described in Barnet *et al.* (*5*) were followed as closely as possible. Electrode placements were C_z, C_3, C_4, O_z, O_1, and O_2, referred to joined mastoids [H. H. Jasper, *Electroencephalogr. Clin. Neurophysiol.* **10**, 371 (1958)]. The EEG was amplified by means of an Elema Schonander EEG machine with half-amplitude bandpass of 0.7 to 70 Hz.
15. All but 11 of the evoked potentials were recorded during non-rapid eye movement sleep. The results were similar whether or not the 11 REM evoked potentials were included in the analyses; see also (*16*).
16. We found that sleep stages in the marasmic subjects could be scored by conventional criteria [T. Anders, R. Emde, A. Parmelee, Eds., *A Manual of Standardized Terminology, Techniques and Criteria for Scoring of States of Sleep and Wakefulness in Newborn Infants* (Neurological Information Network, National Institute of Neurological Diseases and Stroke, Los Angeles, 1971); A. Rechtschaffen and A. Kales, Eds. *A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep States in Human Subjects* (No. 204, U.S. Public Health Service, Washington, D.C., 1968)]. Background EEG's often showed abnormalities including slowing, reduced amplitude, and decreased sleep spindles; or very-high-amplitude fast activity. (Patients whose EEG's showed paroxysmal abnormalities were excluded from the study.) Background EEG and sleep stage interacted with evoked potential characteristics (M. Shkurovich *et al.*, in preparation), but did not fully account for the observed EPI abnormalities. For example, the extremely large N_1P_2 and P_2N_2 components characteristic of the evoked potentials of many marasmic subjects at follow-up were found in all sleep stages. Evoked potentials with high EPI's were often found in "typical" stage 2 or 3 sleep.
17. Norms were based on Barnet *et al.* (*5*). These norms were validated in a subsequent study of 254 evoked potentials [Ohlrich *et al.* (*6*)]. The rules followed for identifying the peaks are given in Barnet *et al.* (*5*). In instances where abnormal evoked potential characteristics made identification of peaks uncertain, the peaks chosen were the ones which minimized the EPI, that is, the scoring method tended to underemphasize deviance.
18. In two previous, similarly conducted evoked potential studies of normal infants [see references in (*17*)] no sex differences were found.
19. We have no information on the heights of the parents of the control subjects from which estimates of genetic growth potential could be made. See Cravioto *et al.* (*2*) and Scrimshaw and Gordon [(*3*), part 2, pp. 16–90] for discussion of height-and-weight-for-age as indicators of nutritional status during infancy and early childhood in developing countries.
20. The evoked potentials were recorded during a study of infant malnutrition being conducted by the Nutrition Service of the Hospital de Niño. Interactions of EPI's with sex and other cofactors will be reexamined later (J. Cravioto *et al.*, in preparation).
21. We thank the personnel of the Servicio de Nutricion, R. Arrieta, chief, for their cooperation. We thank M. Campos for assistance in data collection and J. Auñon, B. Shanks, and B. Barnet for assistance and advice. The research was supported in part by the W. T. Grant Foundation and Public Health Service grants and awards HDO2296 and K2MH45472.

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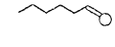
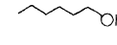
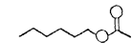
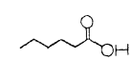
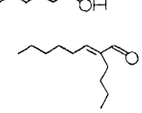
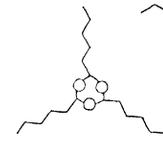
Proteins in a Nonvenomous Defensive Secretion: Biosynthetic Significance

Abstract. *In common with many arthropods, the true bug,* Leptoglossus phyllopus, *when disturbed, emits a two-phase secretion that consists of an organic phase and an aqueous phase. The organic phase is a mixture of highly reactive low-molecular-weight compounds, analogous to those produced by other arthropods, and is deterrent to many kinds of predators. The aqueous phase, heretofore ignored in most analyses of arthropod defensive secretions, contains proteins. Even though the secretion is not injected, the proteins enzymatically catalyze the derivation of the most reactive components within the impermeable cuticular storage reservoir and, thus, constitute part of the defensive system that appears to be commonly used by arthropods producing irritating chemicals.*

Arthropods are conspicuous for emitting chemical irritants for defense against would-be predators (*1*). Natural product chemists have identified such reactive compounds as hydrogen cyanide, quinones, α,β-unsaturated aldehydes, and carboxylic acids in the defensive secretions of arthropods, ranging from millipedes, centipedes, opilionids, and whip scorpions to the phylogenetically advanced cockroaches, walkingsticks, stinkbugs, and beetles (*1–6*). Yet, despite the fact that practically all of the defensive secretions examined closely have been reported to contain an immiscible aqueous phase (*3, 4, 7*), in addition to the reactive organic phase, the chemical analysis of the aqueous phase has been almost totally ignored (*3*). We have found that the defensive secretion of the coreid bug *Leptoglossus phyllopus* contains a mixture of compounds not unlike those identified from other so-called stinkbugs or true bugs in the order Hemiptera (*4–6*). In addition, the aqueous phase contains at least four proteins. The secretion is not injected as are the venoms of some bees, wasps, spiders, snakes, and various marine organisms. We now report that the protein fraction of the secretion catalyzes the production of the most irritating constituents of the defensive blend from a relatively nontoxic precursor within the impermeable cuticular reservoir of the gland.

Adults of *L. phyllopus* were reared in the laboratory, and the volatile components of the defensive secretion from the capacious metathoracic gland were analyzed immediately (Table 1) (*8*). The metathoracic gland in terrestrial Hemiptera (Georcorisae) consists of a large nonglandular storage reservoir lined with cuticle; two pairs of accessory glands, the primary and secondary accessory glands, empty into the reservoir (Fig. 1) (*5, 7*). The secretion released from the reservoir by 1-week-old bugs contained equivalent amounts of hexyl acetate and hexanal (Table 1). In 10-week-old bugs, the amount of hexyl acetate had declined by almost 45 percent, with a corresponding increase in the proportion of hexanal. Comparison of an extract of the primary

Table 1. Composition of the metathoracic gland secretion from 1- and 10-week-old *Leptoglossus phyllopus*.

Compound	Structure	1 week old (%)	10 weeks old (%)
Acetic acid		N.D.*	N.D.*
Hexanal		42.1 ± 8.0	87.8 ± 3.7
1-Hexanol		6.6 ± 1.8	1.2 ± 0.6
Hexyl acetate		49.4 ± 7.7	5.1 ± 3.7
Hexanoic acid		Trace	4.0 ± 4.7
2-*n*-Butyloct-2-enal		N.D.*	N.D.*
Hexanal trimer†		0.2 ± 0.1	2.0 ± 0.6

*Not determined. †Both *cis* and *trans* isomers.

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